



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2021; 10(8): 773-778

© 2021 TPI

www.thepharmajournal.com

Received: 12-05-2021

Accepted: 19-06-2021

Rahul Batheja

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Narayan Ramamurthy

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Ajai Lawrence D' Silva

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Dinesh C Pathak

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Asmita Singh

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Asok Kumar Marriappan

Avian Diseases Section, Division of
Pathology, Indian Veterinary
Research Institute, Izatnagar,
Bareilly, Uttar Pradesh, India

Sohini Dey

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Madhan Mohan Chellappa

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Corresponding Author:

Sohini Dey

Recombinant DNA Laboratory,
Division of Veterinary Biotechnology,
I.V.R.I., Izatnagar, Bareilly, Uttar
Pradesh, India

Chicken anemia virus infection of poultry and its control by vaccines

Rahul Batheja, Narayan Ramamurthy, Ajai Lawrence D Silva, Dinesh C Pathak, Asmita Singh, Asok Kumar Marriappan, Sohini Dey and Madhan Mohan Chellappa

Abstract

Chicken anemia virus (CAV) is a non enveloped virus with icosahedral symmetry. It is one of the smallest viruses having diameter of 25 nm that encloses a single stranded circular negative DNA of 2.3 kb size. CAV has been identified as the causative agent of chicken infectious anemia. Chicken infectious anemia disease is also known by name of Blue wing disease, anemia dermatitis syndrome and hemorrhagic aplastic anemia syndrome based on clinical and histopathological lesions. Clinical signs such as anorexia, weakness, stunting growth, unthriftiness, petechiation, ecchymoses, weight loss, anemia can be observed externally in chicks less than 2 weeks of age. However, adult chickens can also get infected with the virus, though with subclinical symptoms after the maternal antibodies wane. Age related resistance against the disease starts to develop at about 1 week of age and usually gets completed by 2 weeks of age but resistance to infection never develops. Further, CAV once introduced into the flock is considered as a tough virus to deal with because of its resistance to many commonly used disinfectants and its sub-clinical nature in adults and also it is economically devastating to the poultry farmers and hence letting it not into the flock is the only solution. This can easily be achieved by developing highly efficient vaccine against CAV. In view of all the parameters discussed above in this review article we are going to discuss about different types of strategies employed by different research groups for development of high efficacy vaccines against CAV with their advantages and disadvantages. Further we will discuss about what are the future prospect for to increase the efficacy of vaccines.

Keywords: Chicken anemia virus, symmetry, CAV, maternal antibodies

Introduction

In the past few years, reports of various contagious disease outbreaks in poultry are emerging from every corner of world. Some of these diseases are zoonotic in nature and can cause contagious infections to humans also. Especially after the COVID 19 crisis scientific community is keenly monitoring the zoonotic diseases to determine the future risk. Recently one report has emerged from China where a human was found to be infected with H10N3 strain of avian influenza which further implies the need to have an insight into poultry viral diseases. Amongst reports of various disease outbreaks in poultry chicken infectious anemia prevalence reports are more frequent in recent year and it has also been classified as emerging diseases. Further various CAV homologue viruses has been reported from humans population from different regions of world. A CAV homologue was found to be present on human skin surface (HGyV) in 2011 and later in 2012 a similar kind of CAV (JQ690762) was isolated from pediatric fecal samples from China. Another CAV related virus (GyV3) was isolated from fecal samples of Chilean children. A virus named as GyV4 was also reported from children faecal samples in Hong Kong which implies that CAV can be a potential threat to humans. As human homologue of CAV can lead to generation of novel strains by recombination with poultry strains, this necessitates the research community to review the situation related to CAV time often. Considering the importance of CAV is causing a potential future pandemic. This article reviews different types of vaccines developed by against Chicken infectious anemia infection.

Chicken Anemia Virus

Chicken anemia virus (CAV) has been identified as the causative organism of chicken infectious anemia. It is a non enveloped virus with icosahedral symmetry. Thirty two structural subunits arranged in a class P = 3 icosahedron leads to formation of the icosahedral

capsid (McNulty *et al.*, 1990c) ^[17]. CAV is one of the smallest viruses having diameter of 25 nm that encloses a single stranded circular negative DNA of 2.3 kb size (Gelderblom *et al.*, 1989; Noteborn *et al.*, 1991; Todd *et al.*, 1990) ^[2, 11, 17]. CAV genome transcribes with only one promoter which consists of four or five 21 bp direct repeats interrupted by 12 bp sequence which leads to initiation of single polycistronic transcript of 2.1 kb size (Noteborn *et al.*, 1992; Phenix *et al.*, 1994) ^[12, 14]. This polycistronic transcript contains 3 ORFs which are partially overlapping in nature and have a unique property of being polyadenylated approx 25 bases downward from signal site encoding polyadenylation (AAUAAA). CAV is resistant to some harsh chemicals like chloroform, acetone which are used regularly for disinfection and can survive even in highly acidic conditions ranging upto pH 3. Additionally, CAV is found to be very stable at high temperatures can survive at 80°C for 30 min and complete inactivation occurs at 100°C for 10 mins. All these characteristics of CAV leads to its ubiquitous nature. (Goryo *et al.*, 1985; Urlings *et al.*, 1993; Todd *et al.*, 1990) ^[3, 20, 17].

Proteins encoded by CAV

CAV genome encodes three proteins named as VP1, VP2 and VP3 (Noteborn and Koch 1995). VP1 protein is a largest among all encoded proteins having size of 54KDa. It is the only one known to be involved in capsid formation of CAV (Todd *et al.*, 1990) ^[17]. Coding sequence of VP1 is found to be least conserved among all encoded proteins. The N terminal region of VP1 is rich in positive charge amino acids like arginine which assists VP1 protein in binding with DNA. VP2 protein shows a phosphatase activity on serine, threonine and tyrosine phosphates of both VP1 and VP3 and because of this activity it acts as scaffold protein for VP1 to attain its proper conformation. Synchronous synthesis of both VP1 and VP2 proteins in the same cell is must for generation of conformational neutralizing epitope (Noteborn *et al.*, 1998) ^[13] which can be used for development of vaccines against CAV. VP2 also interacts with VP3 in the nucleus and down regulates apoptotic activity by removing phosphate attached to threonine at 108th position (Lai *et al.*, 2017) ^[6]. VP3 is known by the name of apoptin because of its apoptosis inducing property in thymocytes and erythroblasts of young chicken which results in anemia and immunosuppression. It has natural ability to selectively induce apoptosis in rapidly dividing tumor cells.

Clinical and histopathological signs

Chicken infectious anemia is also called as Blue wing disease because of presence of ecchymotic haemorrhages under the wings, anemia dermatitis syndrome and hemorrhagic aplastic anemia syndrome due to intramuscular haemorrhages and aplasia of bone marrow. Primarily CAV infects hemocytoblast cells of the bone marrow and precursor lymphocytes in cortex region of the thymus. As hematopoietic stem cells are precursors of thrombocytes, their deficiency causes thrombocytopenia which leads to intramuscular hemorrhages in chicks (Kuscu and Gurel, 2008) ^[5].

Clinical signs such as anorexia, weakness, stunting growth, unthriftiness, petechiation, ecchymoses, weight loss, anemia can be observed externally while intramuscular haemorrhages, lymphoid atrophy and bone marrow aplasia are mostly seen histopathologically among the young chicks less than 2 weeks of age. Due to anemia comb, eyelids, legs, carcass and wattles gets pale, hematologically, blood becomes

watery with slow clotting properties and in some cases death may also occur (Yuasa *et al.*, 1986) ^[22]. Neurological signs of the disease include depression, dullness and paresis that are visible from outside.

However, adult chickens can also get infected with the virus, though with subclinical symptoms after the maternal antibodies wane. Age related resistance against the disease starts to develop at about 1 week of age and usually gets completed by 2 weeks of age but resistance to infection never develops. There is usually no clinical manifestation of disease or loss of egg production in adult layers but immunosuppression is present which could lead to vaccine failure as well as increase risk of secondary infection. Further, hens can transmit CAV horizontally as well as vertically if chronically infected.

Development of vaccines against CAV

CAV is considered as a tough virus to deal with once introduced in the flock because of its resistance to many commonly used disinfectants and its sub-clinical nature in adults. Further, It is economically devastating to the poultry farmers and hence letting it not into the flock is the only solution. This can easily be achieved by developing highly efficient vaccine against CAV. As clinical disease is only being reported in chicks, chicks can be protected through maternal antibodies obtained vertically from vaccinated breeders which could protect them upto 3 weeks of age from severe clinical signs of CAV (Todd, 2000) ^[18]. After antibodies wane, chicks are still susceptible to this infection though with sub-clinical symptoms which further complicate the problem as they can be still transmitting the disease without showing clinical signs. Presently commercial vaccines are live attenuated type which have proven to be effective against this virus, but has the risk of being transmitted both horizontally and vertically to other chicks. Continuous reports of CAV outbreaks indicating vaccine failure has led to emergence of myriads of modern vaccines with potential protection ability.

Inactivated vaccines

Inactivated vaccines are preferred over live attenuated vaccines because attenuated vaccines can revert back to its virulence after recombination with virulent strain of virus (Sawant *et al.*, 2015) ^[15]. As CAV has widespread prevalence round the globe, possibilities are high that continuous use of live attenuated vaccines can lead to the formation of new strains of CAV. Another problem associated with using attenuated vaccines is its residual pathogenicity. Inactivated vaccines are considered safe in this aspect but drawback of their use is generation of low immune response which could easily be addressed by using high potential immunogenic adjuvants. Taking note of advantages of inactivated vaccine over other types of vaccines to control CAV Zang *et al.*, 2015 developed a novel vaccine against CAV by inactivating highly pathogenic isolate GD-G-12 with propiolactone hydrolysis. The safety prospect of developed inactivated CAV vaccine was evaluated by inoculating 1 mL of the inactivated vaccine (7.9×10^{17} copy/L) in 30 hens. None of the hens showed signs related to CAV. They also conducted pathological studies on three sacrificed hens at 7, 14 and 21 days post inoculation and found out thymus to be normal in all sacrificed birds which further consolidated the finding. Further, the efficacy of the inactivated vaccine was determined by immunizing SPF hens twice at 91 days of age

and 105 days of age by intra-muscular route and antibodies corresponding to CAV were detected in hens serum at interval of 7 days upto 42 days post immunization by ELISA. The results indicated that antibody titres start to rise after first immunization and reached its peak value at 14 days post second immunization. Similar ELISA based antibody evaluation was conducted in chicks hatched from eggs laid by vaccinated hens to determine vertical transmission of antibodies. On first day of hatch antibody titers against CAV was 8518 which got declined to 8398 on 7th day until it got waned subsequently.

DNA based vaccines

DNA vaccines can be the solution to problems associated with attenuated and inactivated vaccines such as inability of CAV to grow to high titres and residual pathogenicity. Here whole virus is not used but a small segment of the viral genome is used which encodes viral protein having neutralizing epitopes. As it is only segment of genome it cannot lead to development of new strains. Another advantage associated with DNA vaccines is they induce wider range of immune response from cell mediated to antibody mediated and have the ability to generate immune responses in the presence of maternal antibodies and are very much tolerated by hosts. A major limitation in using DNA vaccines is that the vaccines does not spread far away from inoculation site and may lead to limited response. In view of reducing the drawbacks associated with DNA vaccines, various research groups have used different strategies to enhance the immune response induced by DNA vaccines.

Moeini *et al.*, 2011^[9, 10] contributed immensely towards development of DNA based vaccines against CAV. They amplified VP1, VP2 genes from CAV isolate SMSC-1 and VP22 from MDV-1. The amplified products were ligated into eukaryotic expression vector, pBudCE4.1 to produce recombinant pBudVP2-VP1 vector containing VP2 and VP1 proteins encoding genes and vector pBudVP2-VP1/VP22 contained the VP2 encoding gene and VP22 gene linked with VP1. VP22 protein of MDV-1 has a distinct characteristic property of migrating between cells and can be useful in increasing the potency of DNA based CAV vaccines if linked with antigenic protein. The expression ability of recombinant pBudCE4.1 vector was analyzed by transfecting MDCC-MSB1 cells with liposomes enclosing the desired plasmids and confirmed transcriptional gene expression by using RT-PCR and translational by Western blot and indirect immunofluorescence. For *In vivo* studies SPF chickens were boosted intra muscularly with different recombinant constructs at 2 weeks interval starting at 14 days of age. 10 days after last booster dose, the muscle immunized was collected after sacrificing the chicks and recombinant gene expression was confirmed by using the same assays used for determination of *in vitro* expression. Antibodies titre studies in serum was conducted by using blocking ELISA that clearly indicated a higher potency with the pBudVP2-VP1/VP22 construct in developing antibody mediated response. They further determined the neutralization potential of these antibodies by VNT assay and found out that pBudVP2-VP1/VP22 vaccinated groups showed higher rate of 1:512 titre in chicks as compared to pBudVP2-VP1 vaccinated chicks. Further cell mediated response was evaluated by cytokine analysis and splenocyte proliferation assay. Cytokine levels of IL-2 and IFN- γ were found to be higher in pBudVP2-VP1/VP22 inoculated group as compared to

pBudVP2-VP1 inoculated chicks. Splenocytes obtained from pBudVP2-VP1/VP22 vaccinated group showed higher stimulation index as compared to pBudVP2-VP1 vaccinated group when induced by VP1. At last the study concluded that pBudVP2-VP1/VP22 had higher cell mediated and humoral immunizing potential as compared to pBudVP2-VP1 because of the additional intercell migrating ability of VP22 protein and this can be used with other DNA based vaccines to enhance immune response.

Similarly in a related work Moeini *et al.*, 2011^[9, 10] amplified VP1 and VP2 genes of CAV by PCR and inserted into pBudCE4.1 to construct pBudVP1 and pBudVP2-VP1. Subsequently, the recombinant plasmids were tested as vaccine candidates in SPF chicks of 2 weeks age. Chicks immunized with the latter showed a higher titer of neutralizing antibodies against CAV, high levels of IL-2, Th1 cytokines and IFN in serum and proliferation of splenocytes after VP1 induction consolidating the finding that recombinant DNA plasmid co-expressing VP1 and VP2 protein has better potential as DNA vaccine as compared to recombinant DNA plasmid that contains only VP1.

Sawant *et al.*, 2015^[15] used different strategy to overcome the drawback of suboptimal immune response generated with DNA vaccines by using HMGB1 C protein as adjuvant. A C terminal deficient HMGB construct was generated using specific primers to reverse transcribe PBMCs. This construct was subsequently expressed in PET expression system and the expressed protein was purified with the help of Ni-NTA column. VP1 and VP2 PCR amplified products were ligated into pTARGET vector to generate a recombinant clone pTARGET+VP1+VP2. *In vitro* expression studies were done by RT – PCR and Western blotting assays *in vitro* cells. For *in vivo* studies, chicks were randomly divided into five groups including vector and negative controls. Primary vaccination of chicks was done at 3 weeks of age and boosted at 2 weeks gap upto 7 weeks of age. Assessment of cell mediated response was carried by determining CD4/CD8 ratio at weekly interval. The study indicated significant higher cell mediated immune response in HMGB co immunized group and least in primary vaccinated only group among all groups. While none of the groups showed any kind of detectable antibody mediated response upto 1st week after booster dose, the HMGB co administered group antibody titre values were higher confirming the immunoenhancing effect of HMGB. It was concluded that pTARGET+VP1+VP2 + HMGB has higher potential to elicit both antibody and cellular mediated immune response and can be future potent vaccine candidate against CAV

Subunit vaccines

The commercially available CAV vaccines have been developed from wild-type CAV strain by passaging serially in cells cultures or chicken embryos. These serially passaged viruses may not be completely attenuated and hence can cause disease in young birds. Subsequently, it can be transmitted to other birds through both horizontal and vertical routes. This problem can be overcome by using subunit vaccines based on CAV structural proteins. Both prokaryotic or eukaryotic expression systems have been used to produce subunit vaccines against CAV.

Subunit vaccines based on prokaryotic expression system

Prokaryotic system is the simplest system to express desired proteins. Among different prokaryotic systems, E.coli host is

frequently used for various purposes. The advantages associated with *E. coli* usage is that it replicate quickly and yields large amounts of protein in less time. Additionally *E. coli* can withstand harsh environment conditions because of the presence of cell wall. *E. coli* is advantageous for expression of cytosolic proteins that require minimum post translational modifications. In view of the advantages associated with prokaryotic systems various research groups have tried to express CAV proteins and determine its immunogenic potential.

Fang *et al.*, 2018 constructed three recombinant plasmids by inserting VP1, VP2 and VP3 PCR amplified genes of CAV in different pET 28 expression vectors and expressed them in *Escherichia coli* system. The expressed VP1, VP2 and VP3 proteins were purified using Ni-NTA column and different combinations of purified proteins were inoculated with CpG-ODN or Freund's immune adjuvants in chicks at 1 week and 3 weeks of age. The humoral immune response in serum was estimated at weekly interval after first immunization for 5 weeks. The ELISA assay results showed that groups inoculated with both VP1 + VP2 combined by CpG-ODN or Freund's immune adjuvants showed serum immuno positivity with in a week of primary immunization but positivity rate in chicks was 20% more in CpG-ODN co administered group as compared to Freund's adjuvant administered group. At 2 weeks post primary immunization all chicks immunized with VP1 + VP2 accompanied by CpG-ODN showed detectable level of antibodies in serum while in other groups a similar rate was obtained only after a week after 3rd week booster dose. It was concluded that CpG-ODN was better adjuvant than Freund's adjuvant and can be used to co inoculate with VP1 + VP2 to induce higher and early antibody positivity rate.

A similar kind of work was also performed by Shen *et al.*, 2015 by expressing VP1 protein of CAV and pigeon IFN γ in *E. coli*. They expressed the N terminal 129 amino acid deleted rVP1 and rPiIFN- γ in *E. coli* expression system and purified them with the help of Ni-NTA column. Purified proteins were inoculated in chicks at 7th and 17th of age individually and with different combinations. Serum was collected at 10 and 14 days post primary inoculation. Using ELISA assay they determined that chicks inoculated with rVP1 and rPiIFN- γ showed highest antibody titer as compared to other groups and similar trend continued even at 21 days of age. They further conducted a real time PCR assay to determine the mRNA levels of IL4 and IFN γ in CAV stimulated splenocytes. For this splenocytes were collected at 21 days of age and were stimulated with CAV for 48 hrs. Real time PCR assay results indicated significant higher level of IFN γ in chicks inoculated with rVP1 and rPiIFN- γ as compared to other groups where as IL4 levels were consistent in all groups. This may be due to stimulation of cell mediated response by IFN γ . It was concluded that IFN γ can be used as an adjuvant to enhance both cell mediated and antibody mediated response and rVP1 and rPiIFN- γ when co inoculated has the potential to be used as subunit vaccine against CAV.

Subunit vaccines based on eukaryotic expression system

Eukaryotic expression system is more difficult to work with than prokaryotic because of their higher generation interval which makes working with them time consuming and is less efficient in terms of amount of protein produced. Additionally eukaryotic system is not easy to deal with and is very

expensive which further complicates the problem. In spite of the drawbacks, proteins are expressed in eukaryotic expression system because of the post translational modifications they impact which helps proteins in attaining their native conformation. It is known that signature properties of some proteins rely mainly on proper conformation and altering this would alter their properties. VP1 protein of CAV is amongst those proteins that exposes its neutralizing conformational epitopes only when it attains its proper conformation. Considering all these advantages several research groups have expressed CAV proteins in eukaryotic expression vectors to determine their immunogenic potential.

Koch *et al.*, 1995 were the first to report that co expression of VP1 and VP2 is must for development of neutralizing antibodies. They proved this by doing virus neutralization assay studies with yolk extract as well as serum. They further confirmed their findings with the help of autopsy. For this they developed three recombinant baculoviruses containing VP1, VP2 and VP3 genes and made them to infect *Spodoptera frugiperda* cells singly as well as in various possible combinations. Cell lysates obtained after infections were inoculated into chicks at 6 weeks of age. They compared the immunogenic potential of mixture of cell lysates prepared by mixing lysate obtained from different individual recombinant baculovirus expressing VP1, VP2 and VP3 infected cells with the cell lysates obtained after co infection with different combinations of recombinant types of baculoviruses. To determine the immunogenicity, they performed VNT assay. Neutralization titre's of mixed lysate group was determined to be 1:32 in 2 out of 8 chicks at 42 days post inoculation while in co infected group the mean titre value reached upto 464 at similar time which indicated that co expression of CAV proteins is desired for obtaining high titres of neutralizing antibodies. They continued their studies in fertilized eggs obtained from immunized hens. By performing VNT studies on yolk extracts they determined that VP1 and VP2 co synthesis is must and sufficient for induction of neutralizing antibodies against CAV. They further conducted the challenge studies by infecting 1 day old chicks with highly virulent CAV-CUX-1 strain. Pathogenicity was determined by autopsy at 6 and 14 days post inoculation and found that thymus was found to be normal in chicks obtained from mothers immunized with cell lysate having co expressing VP1+VP2 or VP1+VP2+VP3 proteins. This further evidenced the finding that VP1 + VP2 co expression in single cell is must for the exposure of neutralizing epitope on VP1.

Noteborn *et al.*, 1998^[13] proved immunologically that VP1 and VP2 proteins co expression is a must for exposure of neutralizing epitopes by VP1. They concluded this by performing immunofluorescent assay as well as immunoprecipitation assay. In immunofluorescent assay they compare neutralizing monoclonal antibodies associated fluorescence between *Spodoptera frugiperda* (Sf9) cells infected individually with VP1 or VP2 expressing recombinant baculovirus to Sf9 cells co-infected with both VP1-and VP2 expressing recombinant baculoviruses or with single baculovirus expressing both VP1 and VP2 proteins and found out that Sf9 cells expressing VP1 and VP2 proteins synchronously using single baculovirus or through different baculoviruses showed better immunofluorescence with neutralizing monoclonal antibodies. They consolidated this finding further by immunoprecipitation studies. When Sf9 cells lysate co expressing both VP1 and VP2 proteins

immunoprecipitated with monoclonal antibodies specific to VP2 protein leads to co precipitation of VP1. This study further confirmed that both VP1 and VP2 proteins interact with each other for a transient period of time.

Tseng *et al.*, 2019^[19] also made recombinant baculovirus to express in *Sf9* cells. The codon optimized VP1 and VP2 proteins of CAV were expressed together in a single construct but through different expression cassette and a single-chain chicken interleukin 12 (chIL-12) was made to express through a separate recombinant baculovirus. The expression of VP1, VP2 and chIL-12 was confirmed by Western blotting and immuno fluorescence assays using anti His antibodies. The bioactivity of recombinant chIL-12 was also confirmed by stimulating secretion of interferon- γ in chicken splenocytes. Furthermore, the ability of VP1 to form virus-like particles (VLPs) in the presence of VP2 was also confirmed by transmission electron microscopy using negative staining. Specific pathogen-free chickens were inoculated at 36 weeks of age with VLPs, VLPs+chIL-12 (5 ng), VLPs + chIL12 (10 ng) and compared their immunogenicity potential at weekly interval. After 1 week post vaccination VLPs + chIL12 (10 ng) inoculated chickens showed significantly higher titres as compared to other groups but at 2 weeks post vaccination titres of both chIL12 inoculated chickens were found to be 3459 and 4570 which then reached to its highest value at 21 days post vaccination of 9034 and 8497. Titre values of both the groups were found to be always higher than positive control group proving their potential as commercial vaccines. Cell-mediated immunity was determined by collecting splenocytes 28 days post vaccination and stimulating them with purified rVP1 in triplicates. The highest stimulation index was obtained in splenocytes collected from VLPs + chIL12 (10 ng) group which further conformed the role of chIL-12 as a strong adjuvant.

Xiao *et al.*, 2001^[21] generated recombinant plasmids pBac-vp1 and pBac-vp2 using pBacPAK8 and VP1 and VP2 and transfected BmN cells with linearized baculovirus Bm-BacPAK6 DNA giving rise to recombinant viruses Bm-vp1 and Bm-vp2. Both the viruses were used to co-infect silkworms to produce recombinant proteins to be used in chickens.

Conclusion

Each type of CAV vaccine has its own advantages as well as disadvantages. It will be advantageous to use different types of vaccines on the basis of different prevailing conditions. For eg. live attenuated vaccine should not be used in a region where CAV is endemic as it could lead to development of new strain. Further, it is difficult to attain high titers of CAV which leads to limited supply of inactivated vaccine. DNA as well as subunit protein vaccines are not found to induce complete immune response individually. The DNA vaccines are inefficient in inducing antibody mediated immune response whereas subunit protein vaccines are inefficient in inducing cell mediated immune response. Hence, it will be prudent to develop a standard regime empirically using both DNA and subunit protein vaccines that provides complete immune response without having much disadvantages.

References

1. Fang L, Zhen Y, Su Q, Zhu H, Guo X, Zhao P. Efficacy of CpG-ODN and Freund's immune adjuvants on antibody responses induced by chicken infectious anemia virus VP1, VP2, and VP3 subunit proteins. *Poultry*

Science 2018;98(3):1121-1126.

2. Gelderblom H, Kling S, Lurz R, Tischer I, Bülow VV. Morphological characterization of chicken anaemia agent (CAA). *Archives of Virology* 1989;109(1, 2):115-120.
3. Goryo M, Sugimura H, Matsumoto S, Umemura T, Itakura C. Isolation of an agent inducing chicken anaemia. *Avian Pathology* 1985;14(4):483-496.
4. Koch G, van Roozelaar DJ, Verschuere CA, van der Eb AJ, Noteborn MH. Immunogenic and protective properties of chicken anaemia virus proteins expressed by baculovirus. *Vaccine* 1995;13(8):763-770.
5. Kuscu B, Gürel A. Lesions in the thymus and bone marrow in chicks with experimentally induced chicken infectious anemia disease. *Journal of Veterinary Science* 2008;9(1):15-23.
6. Lai GH, Lien YY, Lin MK, Cheng JH, Tzen JT, Sun FC *et al.* VP2 of chicken anaemia virus interacts with apoptin for down-regulation of apoptosis through de-phosphorylated threonine 108 on Apoptin. *Scientific Reports*, 2017;7(1):14799.
7. Noteborn M, MH, Koch G. Chicken anaemia virus infection: molecular basis of pathogenicity. *Avian Pathology* 1995;24(1):11-31.
8. McNulty MS. Chicken anaemia agent: a review. *Avian Pathology* 1991;20(2):187-203.
9. Moeini H, Omar AR, Rahim RA, Yusoff K. Development of a DNA vaccine against chicken anemia virus by using a bicistronic vector expressing VP1 and VP2 proteins of CAV. *Comparative Immunology, Microbiology and Infectious Diseases* 2011;34(3):227-236.
10. Moeini H, Omar AR, Rahim RA, Yusoff K. Improving the potency of DNA vaccine against chicken anemia virus (CAV) by fusing VP1 protein of CAV to Marek's disease virus (MDV) Type-1 VP22 protein. *Virology Journal* 2011;8(1):119.
11. Noteborn MH, De Boer GF, Van Roozelaar DJ, Karreman C, Kranenburg O, Vos JG *et al.* Characterization of cloned chicken anemia virus DNA that contains all elements for the infectious replication cycle. *Journal of Virology* 1991;65(6):3131-3139.
12. Noteborn MH, Kranenburg O, Zantema A, Koch G, de Boer GF, van der Eb AJ. Transcription of the chicken anemia virus (CAV) genome and synthesis of its 52-kDa protein. *Gene* 1992;118(2):267-271.
13. Noteborn MH, Verschuere CA, Koch G, Van der Eb AJ. Simultaneous expression of recombinant baculovirus-encoded chicken anaemia virus (CAV) proteins VP1 and VP2 is required for formation of the CAV-specific neutralizing epitope. *Journal of General Virology*, 1998;79(12):3073-3077.
14. Phenix KV, Meehan BM, Todd D, McNulty MS. Transcriptional analysis and genome expression of chicken anaemia virus. *Journal of General Virology* 1994;75(4):905-909.
15. Sawant PM, Dhama K, Rawool DB, Wani MY, Tiwari R, Singh SD *et al.* Development of a DNA vaccine for chicken infectious anemia and its immunogenicity studies using high mobility group box 1 protein as a novel immunoadjuvant indicated induction of promising protective immune responses. *Vaccine* 2015;33(2):333-340.
16. Shen SY, Chang WC, Yi HH, Tsai SS, Liu HJ, Liao PC, Chuang KP. Development of a subunit vaccine

- containing recombinant chicken anemia virus VP1 and pigeon IFN- γ . *Veterinary Immunology and Immunopathology* 2015;167(3, 4):200-204.
17. Todd D, Creelan JL, Mackie DP, Rixon F, McNulty MS. Purification and biochemical characterization of chicken anaemia agent. *Journal of General Virology* 1990;71(4):819-823.
 18. Todd D. Circoviruses: immunosuppressive threats to avian species: a review. *Avian Pathology*, 2000;29(5):373-394.
 19. Tseng TY, Liu YC, Hsu YC, Chang PC, Hsieh MK, Shien JH *et al.* Preparation of Chicken Anemia Virus (CAV) Virus-Like Particles and Chicken Interleukin-12 for Vaccine Development Using a Baculovirus Expression System. *Pathogens* 2019;8(4):262.
 20. Urlings HAP, De Boer GF, Van Roozelaar DJ, Koch G. Inactivation of chicken anaemia virus in chickens by heating and fermentation. *Veterinary Quarterly* 1993;15(3):85-88.
 21. Xiao QL, Zhang ZF, He JL. Simultaneous expression of chicken anaemia virus proteins VP1 and VP2 in silkworms. *Sheng Wu Gong Cheng Xue Bao*, 2001;17(3):283-287.
 22. Yuasa N, Imai K. Pathogenicity and antigenicity of eleven isolates of chicken anaemia agent (CAA). *Avian Pathology* 1986;15(4):639-645.